

What is claimed is:

1. A method of diagnosing a cellular proliferative disorder of breast tissue in a subject comprising determining the state of methylation of one or more nucleic acids isolated from the subject, wherein the state of methylation of one or more nucleic acids as compared with the state of methylation of one or more nucleic acids from a subject not having the cellular proliferative disorder of breast tissue is indicative of a cellular proliferative disorder of breast tissue in the subject.
2. The method of claim 1, wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, RAR $\beta$ 2, WT1, HOXA5, 14.3.3 sigma, estrogen receptor, NES-1, and combinations thereof.
3. The method of claim 1, wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, WT1, HOXA5, and combinations thereof.
4. The method of claim 2, wherein the state of methylation of the nucleic acid(s) is hypermethylation as compared with the state of methylation of the nucleic acid(s) from a subject not having the disorder of breast tissue.
5. The method of claim 2, wherein the methylation of the nucleic acid is in the regulatory region of the nucleic acid or in the coding region of the nucleic acid.
6. The method of claim 2, wherein the nucleic acid isolated from the subject is obtained from blood, plasma, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid, breast tissue, lymph nodes or bone marrow.
7. The method of claim 6, wherein the duct cells are obtained by a procedure selected from ductal lavage, sentinel node biopsy, fine needle aspirate, routine operative breast endoscopy, nipple aspiration and core biopsy.
8. The method of claim 2, wherein the disorder of the breast is selected from the group consisting of ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ*, and papillary carcinoma *in situ*.

9. The method of claim 2, wherein determining the state of methylation comprises amplifying the nucleic acid by means of at least one sense primer and at least one antisense primer that distinguishes between methylated and unmethylated nucleic acids.
10. The method of claim 9, wherein the primers hybridize with target polynucleotide sequences selected from SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82 and combinations thereof.
11. The method of claim 9, wherein the primers are selected from SEQ ID NO:7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90 and combinations thereof.
12. The method of claim 2, further comprising contacting the nucleic acid with a methylation-sensitive restriction endonuclease.
13. The method of claim 12, wherein the methylation-sensitive restriction endonuclease is selected from the group consisting of MspI, HpaII, BssHII, BstUI and NotI.
14. A method of determining a predisposition to a cellular proliferative disorder of breast tissue in a subject comprising determining the state of methylation of one or more nucleic acids isolated from the subject,
 

wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, RAR $\beta$ 2, HOXA5, WT1, 14.3.3 sigma, estrogen receptor, NES-1 and combinations thereof; and

wherein the state of methylation of the nucleic acid(s) as compared with the state of methylation of the nucleic acid from a subject not having a predisposition to the cellular proliferative disorder of breast tissue is indicative of a cellular proliferative disorder of breast tissue in the subject.
15. The method of claim 14, wherein the state of methylation of the nucleic acid(s) isolated from the subject is hypermethylation as compared with the state of methylation of the nucleic acid(s) from a subject not having a predisposition to the disorder of breast tissue.
16. The method of claim 14, wherein methylation of the nucleic acid(s) is in the regulatory region of the nucleic acid(s).

17. The method of claim 14 wherein the nucleic acid(s) isolated from the subject is obtained from blood, plasma, breast tissue, lymph, duct cells, ductal lavage fluid, nipple aspiration fluid or bone marrow.
18. The method of claim 17, wherein the duct cells are obtained by a procedure selected from the group consisting of ductal lavage, sentinel node biopsy, fine needle aspirate, routine operative breast endoscopy, nipple aspiration and core biopsy.
19. The method of claim 14, wherein the disorder of the breast is selected from the group consisting of ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ*, and papillary carcinoma *in situ*.
20. The method of claim 14, wherein determining the state of methylation comprises amplifying the nucleic acid(s) by means of at least one sense primer and at least one antisense primer that distinguishes between methylated and unmethylated nucleic acid.
21. The method of claim 20, wherein the primers hybridizes with target polynucleotide sequences selected from SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82, and combinations thereof.
22. The method of claim 20, wherein the primers are selected from SEQ ID NO:7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90 and combinations thereof.
23. The method of claim 14, further comprising contacting the nucleic acid with a methylation-sensitive restriction endonuclease.
24. The method of claim 23, wherein the methylation-sensitive restriction endonuclease is selected from the group consisting of MspI, HpaII, BssHII, BstUI and NotI.

25. A method for diagnosing a cellular proliferative disorder of breast tissue in a subject comprising:
  - (a) contacting a nucleic acid-containing specimen from the subject with an agent that provides a determination of the methylation state of nucleic acids in the specimen, and
  - (b) identifying the methylation state of at least one region of at least one nucleic acid, wherein the methylation state of at least one region of at least one nucleic acid that is different from the methylation state of the same region of the same nucleic acid in a subject not having the cellular proliferative disorder is indicative of a cellular proliferative disorder of breast tissue in the subject.
26. The method of claim 25, wherein the regions of the nucleic acid are contained within CpG-rich regions.
27. The method of claim 25, wherein the methylation state of at least one region of at least one nucleic acid from the subject comprises hypermethylation when compared to the same region(s) of the nucleic acid in a subject not having the cellular proliferative disorder.
28. The method of claim 27, wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, RAR $\beta$ 2, HOXA5, WT1, 14.3.3 sigma, estrogen receptor, NES-1 and combinations thereof.
29. The method of claim 27, wherein the nucleic acid is selected from the group consisting of Twist, cyclin D2, HOXA5, NES-1 and WT1.
30. The method of claim 27, wherein the agent is at least one sense primer and at least one antisense primer that hybridize with a target sequence in the nucleic acid.
31. The method of claim 30, wherein the target nucleic acid sequence is selected from SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82, and combinations thereof.
32. The method of claim 30, wherein the primers are selected from the group consisting of SEQ ID NO:7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90 and combinations thereof.

33. The method of claim 27, wherein the specimen is selected from blood, plasma, breast tissue, biopsy sample, lymph, lymph node, ductal lavage, nipple aspiration fluid and bone marrow.
34. The method of claim 27, wherein the disorder of the breast is selected from ductal carcinoma *in situ*, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma *in situ*, lobular carcinoma *in situ*, and papillary carcinoma *in situ*.
35. A kit for the detection of a cellular proliferative disorder of breast tissue in a subject comprising
- (a) carrier means compartmentalized to receive a nucleic acid-containing sample from the subject therein;
  - (b) a reagent that modifies unmethylated cytosine nucleotides
  - (c) at least one sense primer and at least one antisense for amplification of CpG-containing nucleic acid, wherein the primers can distinguish between modified methylated and non-methylated nucleic acid.
36. The kit of claim 35, wherein the primers hybridize with a target polynucleotide sequence selected from the group consisting of SEQ ID NO:1-4, 15-18, 25-36, 41-48, 65-66, 73-76, 81-82, and combinations thereof.
37. The kit of claim 35, wherein the primers are selected from the group consisting of SEQ ID NO:7-14, 21-24, 37-40, 49-64, 69-72, 77-80, 85-90 and combinations thereof.

SEQ ID NO:	Gene	sense/antisense		
1	WT	sense	5'-GCGGCGCAGTTCCCCAACCA-3'	nucleotides 882-901
2	WT	antisense	5'-ATGGTTTCTCACCAGTGTGCTT-3'	nucleotides 1416-1437
3	WT	sense	5'-GCATCTGAAACCAGTGAGAA-3'	nucleotides 1320-1339
4	WT	antisense	5'-TTTCTCTGATGCATGTTG-3'	nucleotides 1685-1702
5	WT	sense	5'-GATTGGCTACCCAAGTGTGCA-3'	
6	WT	antisense	5'-CAGGGGCAGCAGCCACAAAGGC-3'	
7	WT	sense	5'-TTTGGGTAAAGTTAGGCGTCGTCG-3'	
8	WT	antisense	5'-ACACTACTCCTCGTACGACTCCG-3'	
9	WT	sense	5'-TTTGGGTAAAGTTAGGTGTTGTTG-3'	
10	WT	antisense	5'-ACACTACTCCTCATACAACTCCA-3'	
11	WT	sense	5'-CGTCGGGTGAAGGCGGGTAAT-3'	
12	WT	antisense	5'-CGAACCCGAACCTACGAAACC-3'	
13	WT	sense	5'-TGTTGGGTGAAGGTGGGTAAT-3'	
14	WT	antisense	5'-CAAACCCAAACCTACAAAACC-3'	
15	cyclin D2	sense	5'-CATGGAGCTGCTGTGCCACG -3'	
16	cyclin D2	antisense	5'-CCGACCTACCTCCAGCATCC -3'	
17	cyclin D1	sense	5'-AGCCATGGAACACCAGCTC-3'	
18	cyclin D1	antisense	5'-GCACCTCCAGCATCCAGGT-3'	
19	cyclin D2	sense	5'-GATTGGCTAC CCAACTGTTGCA-3'	
20	cyclin D2	antisense	5'-CAGGGGCAGCAGCCACAAAGGC-3'	
21	cyclin D2	sense	5'-GTTATGTTATGTTTGTGTTGTATG-3'	unmethylated
22	cyclin D2	antisense	5'-GTTATGTTATGTTTGTGTTGTATG-3'	unmethylated
23	cyclin D2	sense	5'-TACGTGTTAGGGTCGATCG-3'	methylated
24	cyclin D2	antisense	5'-CGAAATATCTACGCTAAACG-3'	methylated
25	14.3.3 sigma	sense	5'-ACAGGGGAACTTTATTGAGAGG-3'	A 375 bp $\sigma$ -specific probe
26	14.3.3 sigma	antisense	5'-AAGGGCTCCGTGGAGAGGG-3'	(SEQ ID NO:26)
27	14.3.3 sigma	sense	5'-GAGGAGTGTCCCGCCTTGTGG-3'	A TG repeat sequence in the

SEQ ID NO:	Gene	sense/antisense		
				3'UTR of $\sigma$
28	14.3.3 sigma	antisense	5'- GTCTCGGTCTTGCACTGGC3'	
29	14.3.3 sigma	sense	5'-GTGTGTCCCCAGAGCCATGG-3'	A 1.2 kb PCR product, encompassing the entire $\sigma$ coding sequence, was generated using two primers
30	14.3.3 sigma	antisense	5'- GTCTCGGTCTTGCACTGGCG-3'	(antisense; SEQ ID NO:30
31	14.3.3 sigma	antisense	5'-CACCTTCTCCCGGTACTCACG-3'	entire $\sigma$ coding sequence:
32	14.3.3 sigma	sense	5'-GAGCTCTCCTGCGAAGAG-3'	entire $\sigma$ coding sequence:
33	14.3.3 sigma	sense	5'-GAGGAGGCCATCCTC TCTGGC-3'	entire $\sigma$ coding sequence:
34	14.3.3 sigma	antisense	5'-TCCACAGTGTCAGGTTGTCTCG-3'	entire $\sigma$ coding sequence:
35	14.3.3 sigma, first exon	sense	5'-GAGAGAGTTAGTTTGTATTAGAAAG-3'	start at nt 8641 generates a 474 bp PCR product
36	14.3.3 sigma	antisense	5'-CTT ACTAATATCCATAACCTCC-3'	(antisense primer with start at nt 9114;
37	14.3.3 sigma	sense	5'-TGGTAGTTTTTATGAAAGGCGTC-3'	methylated DNA
38	14.3.3 sigma	antisense	5'-CCTCTAACCGCCACACG-3'	
39	14.3.3 sigma	sense	5'-ATGGTAGTTTTTATGAAAGGTGTT-3'	unmethylated DNA
40	14.3.3 sigma	antisense	5'-CCCTCTAACCACCCACCACA-3'	
41	14.3.3 sigma	sense	5'-GTGTGTCCCCAGAGCCATGG-3'	PCR was performed using the $\sigma$ -specific primers
42	14.3.3 sigma	antisense	5'-ACCTTCTCCCGGTACTCACG-3'	
43	RAR $\beta$	sense	5'-AGA GTT TGA TGG AGTTGG GTG GAG-3'	227 bp probe was amplified
44	RAR $\beta$	antisense	5'-CAT TCG GTT TGGGTC AAT CCA CTG-3'	

SEQ ID NO:	Gene	sense/antisense		
45	RAR $\beta$	sense	5'-CAGCCCGGGTAGGGTTCACC-3'	W3
46	RAR $\beta$	antisense	5'-CCGGATCCTACCCCGACGG-3'	W3
47	RAR $\beta$	sense	5'-CCGAGAACGCGAGCGATCC-3'	W4
48	RAR $\beta$	anti-sense	5'-GGCCAATCCAGCCGGGGCG-3'	W4
49	RAR $\beta$	sense	5'-GTG GGT GTA GGT GGA ATA TT-3'	unmethylated DNA were as follows: U1
50	RAR $\beta$	antisense	5'-AAC AAA CAC ACA AAC CAA CA-3'	U1
51	RAR $\beta$	sense	5'-TGT GAG TTA GGA GTA GTG TTTT-3'	U2
52	RAR $\beta$	antisense	5'-TTC AAT AAA CCC TAC CCA-3'	U2
53	RAR $\beta$	sense	5'-TTA GTA GTT TGG GTA GGGTTT ATT-3'	U3
54	RAR $\beta$	antisense	5'-CCA AAT CCT ACC CCAACA-3'	U3
55	RAR $\beta$	sense	5'-GAT GTT GAG AAT GTGAGT GAT TT-3'	U4
56	RAR $\beta$	antisense	5'-AAC CAA TCC AACCAA AAC A-3'	U4
57	RAR $\beta$	sense	5'-AGC GGGCGT AGG CGG AAT ATC-3'	methyated M1
58	RAR $\beta$	antisense	5'-CAACGA ACG CAC AAA CCG ACG-3'	M1
59	RAR $\beta$ RAR $\beta$	sense	5'-CGT GAG TTA GGA GTA GCG TTT C-3'	M2
60	RAR $\beta$	antisense	5'-CTT TCG ATA AAC CCT ACC CG-3'	M2
61	RAR $\beta$	sense	5'-GGT TAG TAG TTC GGG TAG GGTTA TC-3'	M3
62	RAR $\beta$	antisense	5'-CCG AAT CCT ACC CCGACG-3'	M3
63	RAR $\beta$	sense	5'-GTC GAG AAC GCG AGCGAT TC-3'	M4
64	RAR $\beta$	antisense	5'-CGA CCA ATC CAA CCGAAA CG-3'	M4
65	RAR $\beta$	sense	5'-GAC TGT ATG GAT GTTCTG TCA G-3'	RT $\pm$ PCR exon 5
66	RAR $\beta$	antisense	5'-ATT TGTCCT GGC AGA CGA AGC A-3'	exon 6
67	actin	sense	5'-ACC ATG GAT GAT GAT ATCG-3'	RT $\pm$ PCR
68	actin	antisense	5'-ACA TGG CTG GGG TGTTGA AG-3'	
69	HOXA5	sense	5'TTTAGCGGTGGCGTTTCG-3'	methyated DNA
70	HOXA5	antisense	5'-ATACGACTTCGAATCACGTA-3'	
71	HOXA5	sense	5'-TTGGTGAAGTTGGGTG-3'	unmethylated
72	HOXA5	antisense	5'AATACAACCTTCAAATCACATAC-3'	
73	HOXA5	sense	5'ATTTTGTATAATGGGTGTAAT3'	
74	HOXA5	antisense	5'AACATATACTTAATTCCCTCC-3'	
75	HOXA5	sense	5' TCATTTTGCGGTCGCTATCC-3'	RT-PCR
76	HOXA5	antisense	5'GCCGGCTGGCTGTACCTG-3'	
77	NES-1	sense	5'-TTGTAGAGGTGGTGTGTTT-3'	unmethylated
78	NES-1	antisense	5'-TTGTAGAGGTGGTGTGTTT-3'	
79	NES-1	sense	5'-TTCGAAGTTTATGGCGTTTC-3'	methyated
80	NES-1	antisense	5'-TTATTTCCGCAATACGCGAC-3'	

SEQ ID NO:	Gene	sense/antisense		
81	NES-1	sense	5'-ACCAGAGTTGGGTGCTGAC-3'	
82	NES-1	antisense	5'-ACCTGGCACTGGTCTCCG-3'	
83	36B4	sense	5'-GATTGGCTACCCAAGTGTGCA-3'	
84	36B4	antisense	5'-CAGGGGCAGCAGCCACAAAGGC-3'	
85	Estrogen Receptor	sense	G GGTGTTTTTG AGATTGTTGG	Unmethylated
86			TG AGTTGTGATG GGTTTTGG	
87		antisense	CCAAAACC CATCACAAGT CA	
88		sense	AGAGTAGGCG GCGAGCGT	methylated
89			CGGGAAAAG TACGTGTTCG T	
90		antisense	A CGAACACGTA CTTTCCCG	